Effect of highly active antiretroviral therapy (HAART) on pharmacokinetics and pharmacodynamics of doxorubicin in patients with HIV-associated non-Hodgkin’s lymphoma

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Background: We demonstrated that highly active antiretroviral therapy (HAART) increases the toxic effect of cyclophosphamide, vincristine, doxorubicin (DOX) and prednisone (CHOP) in HIV-patients with non-Hodgkin’s lymphoma (NHL). To ascertain the cause of increased toxicity, we investigated the pharmacokinetics of DOX in HIV-patients with NHL treated with CHOP with and without HAART.

Methods: Complete pharmacokinetics and pharmacodynamic analysis was determined in 19 patients during 38 cycles of chemotherapy: 19 cycles with CHOP and 19 CHOP+HAART in a crossover-designed study. HAART included protease inhibitors indinavir (IDV) in nine patients, saquinavir (SQV) hard gel in six patients and nelfinavir (NFV) in four patients.

Results: No significant effects of HAART on pharmacokinetics parameters of DOX were observed. Similarly, no differential effect on DOX pharmacokinetics among IDV, SQV, and NFV was evidenced. Significant associations ($P=0.012$) were observed between DOX AUC0–1 (area under the concentration curve) and G3-G4 WHO haematologic toxicity, in patients treated with CHOP alone, but not in those treated with CHOP+HAART ($P=0.012$).

Conclusion: We demonstrated that HAART therapy has no significant effect on DOX pharmacokinetics. DOX AUC appears to be a predictor of toxicity only in patients treated with CHOP alone. Other factors beside DOX plasma levels are detrimental for toxicity after CHOP+HAART. Therefore, pharmacodynamic interactions between HAART and DOX should be considered.

Key words: CHOP, doxorubicin, HAART, HIV, interactions, pharmacokinetics

Introduction

Non-Hodgkin’s lymphoma (NHL) has become the most common AIDS-associated cancer among HIV-positive subjects receiving highly active antiretroviral therapy (HAART) [1].

Chemotherapy regimes based on cyclophosphamide, doxorubicin (DOX) vincristine, and prednisone (CHOP) represent a pivotal treatment for patients with HIV-NHL [2]. Recently, to overcome the complications of HIV infection that could compromise or delay antineoplastic treatment, HAART has often been given in combination with CHOP [3, 4]. Such association results are effective and may contribute in reducing morbidity in HIV-NHL patients. However, the management of severe toxicity observed with CHOP+HAART represents the main limitation of the wide use of this type of combination therapy [4, 5]. It was believed that such unexpected toxicity could be related to pharmacokinetic interactions between antiretroviral and antineoplastic drugs leading to an increased plasma drug exposure. HAART consists of a combination of nucleoside analogue reverse transcriptase inhibitors (NRTIs), with protease inhibitors (PIs) and non-nucleoside analogue reverse transcriptase inhibitors (NNRTIs). PIs, as well as the anticancer drugs in the CHOP regimen, are metabolized by the liver cytochrome P450 CYP3A4 isoform [6]. The metabolic clearance of anticancer drugs sharing this common enzymatic pathway of PI can be inhibited by concomitant administration of PI. In vitro studies demonstrated that PI could also interfere with the activity of P-glycoprotein (Pgp) or multi-drug resistance proteins (MRP), which are involved in cellular efflux of a broad range of drugs including anthracyclines [7, 8]. PIs could interfere with the activity of these carrier proteins present in biliary and renal tracts, resulting in reduced biliary and renal excretions of the antineoplastic drugs (pharmacokinetic interactions). Moreover,
competitive inhibition of Pgp and MRP activity by PI could determine an increased amount of intracellular concentration of antineoplastic drugs in normal cells over expressing Pgp/MRP, resulting in increased toxic side-effects under the same conditions of drug area under curve (AUC) (pharmacodynamic interactions). Pharmacokinetic interactions have been reported between PI and a wide class of drugs used in the clinical management of HIV [9], but only little and incomplete data have been reported on pharmacokinetic interactions between PI and antineoplastic drugs [10, 11].

In order to gain new insight on potential pharmacokinetic interactions between PIs and anticancer drugs and to ascertain the pharmacological basis of the increased toxicity of CHOP, after PI-based HAART combination, we planned a prospective clinical trial in NHL patients who underwent CHOP with or without HAART combination. This study aims to investigate the effect of HAART on DOX pharmacokinetics and the relationship between DOX plasma concentrations and toxicity.

### Patients and methods

#### Patients

All the Caucasian patients with NHL were enrolled in a prospective study at the National Cancer Institute, Aviano, Italy, as already reported [12]. The demographic and clinical characteristics of the 19 patients that participated in the pharmacokinetic study are listed in Table 1. Toxicities were graded using the WHO criteria [13]. The local ethical committee approved the clinical and the pharmacokinetic protocol and all the patients recruited in the study signed an informed consent. Sixteen patients had previously received antiretroviral therapy for at least 1 month (range 1–17 months) including zidovudine (AZT), lamivudine (3TC) and NNRTIs (Table 1).

#### Study design

The patients enrolled in this study received first line NHL chemotherapy, consisting of the following drug regimes. CHOP: cyclophosphamide 750 mg/m² i.v., day 1; adriamycin 50 mg/m² i.v., day 1; vincristine 1.4 mg/m² (max 2 mg) i.v., day 1; prednisone 100 mg p.o., days 1–5; to be repeated every 3 weeks. HAART for HIV treatment was performed with saquinavir hard gel (SQV-HG), 600 mg/day, or nelfinavir (N VF), 800 mg/day, or IDV 800 mg/day in combination with two NRTIs. All patients received the first or the second course of CHOP in the presence or absence of HAART. Patients who started with CHOP crossed over to CHOP+HAART in the second course of chemotherapy (Scheme A → B), whereas patients starting the first cycle of chemotherapy with CHOP without HAART combination. This study aims to investigate the pharmacokinetics of DOX during the cycle with CHOP alone, the patients were previously washed out from PI for 3 days before DOX infusion.

#### Sampling

Blood samples (5 ml) were drawn from peripheral vein and collected in heparinized glass tubes after 15 min infusion with DOX dose (time 0) and at 0.25, 0.5 1, 2, 3, 4, 5, 6, 12, 24 and 48 h from the start of i.v. DOX administration. Blood specimens were immediately centrifuged at 4°C. Plasma samples were collected and heated at 56–58°C for 60 min to inactivate HIV. After inactivation, all samples were stored at −80°C until drug analysis.

#### Drug assay

Plasma level of DOX was assayed by a reversed-phase HPLC method with fluorescence detection adapted from previously reported analytical methods [14]. The method was calibrated by daily standard curves that ranged between 1 and 1000 ng/ml: intraday and interday variability were <10% within this range of concentrations.

#### Pharmacokinetic analyses

Non-compartmental analysis was used to determine DOX pharmacokinetic parameters. The terminal apparent first-order elimination rate (β) was estimated by least squares log-linear fit to the terminal elimination phase. The elimination half-life (t1/2) was calculated by the equation 0.693/β.
The pharmacokinetic parameters of the different groups were compared using Wilcoxon non-parametric test for paired data and the Mann–Whitney test for unpaired data. All comparisons were considered significant at \( P < 0.05 \).

Results

Twenty-one patients were enrolled and complete pharmacokinetic studies of DOX alone and in combination with HAART were determined in 19 patients. Two patients were excluded from the study, one because of progressive disease after the first course of CHOP and the other refused to continue the therapy after a first course of CHOP + HAART. The mean age of the patient population, including three females and 16 males, was 41 years (range 28–63 years). The characteristics of the patients are shown in Table 1. Among the 19 eligible patients, the first course of the CHOP regimen was administered without HAART in 10 patients and with HAART in nine patients. In the second cycle of therapy, the 10 patients treated with CHOP crossed over to CHOP + HAART and the nine patients treated with CHOP + HAART crossed over to CHOP. The PI component of the HAART regimen consisted of SQV-HG in six patients, IDV in nine patients and NFV in four patients, respectively. No significant differences in DOX pharmacokinetic parameters (\( C_{\text{max}}, AUC_{48}, AUC_{0-\infty}, \text{Cl} \) and last \( t_{1/2} \)) as a consequence of HAART were observed. Data are shown in Table 2.

The effect of single PI was also investigated separately in patients treated with CHOP ± SQV-HG, CHOP ± IDV and CHOP ± NFV, respectively. No significant difference due to the single PI was observed (Table 2).

Statistical analysis

The pharmacokinetic parameters of the different groups were compared using Wilcoxon non-parametric test for paired data and the Mann–Whitney test for unpaired data. All comparisons were considered significant at \( P < 0.05 \).

The AUC\(_{0-48}\) for concentration (\( C \)) versus time (\( t \)) was calculated by the linear trapezoidal method from 0 to \( t_{48} \). The total AUC extrapolated to infinity (AUC\(_{0-\infty}\)) was calculated as follows: AUC\(_{0-\infty} = AUC_{0-48} + C_0/t_1\) where total body clearance (\( \text{Cl} \)) and volume of distribution (\( \text{Vd} \)) were determined as Cl = dose/AUC\(_{0-\infty}\) and Vd = Cl/\( b \), respectively.

Discussion

We previously demonstrated that myelosuppression and non-haematological toxicity such as mucosal and neurotoxicity are significantly more frequent in patients treated with a HAART and chemotherapy combination [4]. The correct clinical
management of chemotherapy plus HAART combination implies a deeper knowledge of the basis of such toxicity potentiation and a definition of whether pharmacodynamic interactions between antiretroviral and antineoplastic drugs are a consequence of pharmacokinetic interactions. In this study, we investigated the effect of HAART on the pharmacokinetics of DOX, which represents the most important antineoplastic drug of the CHOP regimen. No significant effect of HAART on DOX pharmacokinetic parameters (AUC, Cmax, t1/2, Cl or Vd) was found.

It was believed that the PI used in the HAART regimen could interfere with anthracycline transport, being either PI or DOX common substrates for cellular transporters Pgp or MRP [15, 16]. However, our study did not highlight any effect of PI on DOX pharmacokinetics, even when the subgroup of patients treated with SQV-HG and NFV, who exhibited a relatively high affinity for Pgp compared with IDV, was considered [15]. Competitive inhibition of cytochrome P450 between PI and DOX could also be hypothesized, but involvement of P450 in DOX metabolism is marginal since the most important metabolic pathway of DOX is represented by the side-chain carbonic reduction at C13, which is catalysed by ubiquitous aldo-keto-reductase to form the (S)-secondary alcohol [17].

Previous studies have demonstrated that plasma concentrations of PI achievable in patients with the schedule we adopted is in the micromolar range (1–10 µM) [18, 19], effective enough to reverse multidrug resistance in in vitro experimental models. Although such plasma concentrations are unable to modulate DOX pharmacokinetics, they are potentially effective in blocking Pgp or MRP activity in normal non-neoplastic cells of patients overexpressing MDR proteins, such as haemopoietic stem cells [20]. This could result in an intracellular enhancement of DOX uptake in normal cells, leading to an increased toxicity due to pharmacodynamic interactions. Interestingly, data from our study evidenced a stronger association between plasma level of DOX AUC and haematological toxicity in the course of CHOP alone. Such association was lacking during the course of CHOP + HAART. This indicates that the toxic effect of DOX + HAART is due to additional factors, probably an increased DOX uptake in normal cells, rather than DOX plasma concentrations. These factors are able to circumvent the effect of DOX AUC, making it less detrimental for toxicity as in the case in which DOX is administered without HAART.

A greater number of patients treated with a specific PI in association with DOX must be examined before drawing final conclusions on pharmacokinetic interactions between DOX and SQV-HG, IDV or NFV, respectively. Moreover, different pharmacokinetic interactions could occur between the other antineoplastic drugs used in the CHOP regimen, such as vincristine or cyclophosphamide and PI. However, our study is one of the largest published, so far, on the effect of HAART within the same patient. Therefore, we are confident that our data could add new knowledge to the understanding of pharmacological interactions between HAART and DOX, thus contributing to improved combination therapies in patients with AIDS-related cancers.

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References


